A MICROFABRICATED FLUIDIC DEVICE FOR FRAGMENTATION

The present invention relates to a microfluidic device
for nucleic acid fragmentation. The device may be used in

5   or conjunction with a microfabricated reaction chamber
system for carrying out, for example, a nucleic acid
sequence amplification and detection process on a nucleic
acid sample.

10   Random fragmentation of DNA or RNA is often necessary
as a sample pretreatment step for, for example, nucleic acid
analysis or genomic library generation. Fragmentation may
be achieved biochemically using restriction enzymes, or
through application of a physical force to break the

15   molecules (see, for example, P. N. Hengen, Trends in
Biochem. Sci. , vol. 22, pp. 273- 274, 1997 and P. F.
Davison, Proc. Nat. Acad. Sci. USA , vol. 45, pp. 1560-
1568, 1959).

20   DNA fragmentation by shearing usually involves passing
the sample through a short constriction. Assuming a
constant volumetric flow rate, the flow velocity increases
rapidly at the constriction inlet. The coiled DNA molecules
unfurl as a result, straightening out along the direction of

25   flow. Whether a stretched molecule actually breaks in half
or depends not only on flow rate, but also on molecule
length and constriction cross-section. DNA can be sheared
by pushing it through a syringe needle. A more complex
instrument relies on an HPLC pump to push a sample

30   repeatedly through a closed loop containing an orifice, with
10 or more cycles yielding fragments as small as 300 bp.

By the term microfabricated device or system as used
herein is meant any device manufactured using processes that
are typically, but not exclusively, used for batch
production of semiconductor microelectronic devices, and in
5   recent years, for the production of semiconductor
micromechanical devices. Such microfabrication technologies
include, for example, epitaxial growth (eg vapour phase,
liquid phase, molecular beam, metal organic chemical vapour
deposition), lithography (eg photo-, electron beam-, x-ray,
10 ion beam-), etching (eg chemical, gas phase, plasma),
electrodeposition, sputtering, diffusion doping and ion
implantation. Although non-crystalline materials such as
glass may be used, microfabricated devices are typically
formed on crystalline semiconductor substrates such as
15 silicon or gallium arsenide, with the advantage that
electronic circuitry may be integrated into the system by
the use of conventional integrated circuit fabrication
techniques. Combinations of a microfabricated component
with one or more other elements such as a glass plate or a
20 complementary microfabricated element are frequently used
and intended to fall within the scope of the term
microfabricated used herein. Also intended to fall within
the scope of the term microfabricated are polymeric replicas
made from, for example, a crystalline semiconductor
25 substrate.

The isolation and purification of DNA and/or RNA from
bacterial cells and virus particles is a key step in many
areas of technology such as, for example, diagnostics,
30 environmental monitoring, forensics and molecular biology
research.

Microfabrication is an attractive construction method
for producing devices for carrying out biological processes
for which very small sample volumes are desirable, such as
DNA sequence analysis and detection.

One such device, for carrying out a polymerase chain
reaction (PCR) followed by a detection step is disclosed in
US 5,674,742. Lamb wave pumps are used to transport DNA
primers, polymerase reagents and nucleotide reagents from
three separate storage chambers into a single reaction
chamber as and when required to carry out a PCR process,
with the temperature of the reaction chamber being cycled as
required.

Another microfabricated device, for carrying out a
chemical reaction step followed by an electrophoresis
separation step, is disclosed in Analytical Chemistry 1994,
66, 4127-4132. Etched structures in a silicon substrate
covered by a glass plate provide a reaction chamber and
connections to buffer, analyte, reagent and analyte waste
reservoirs, as well as an electrophoresis column connected
to a waste reservoir.

Nucleic acid sequence-based amplification (NASBA) is a
primer-dependent technology that can be used for the
continuous amplification of nucleic acids in a single
mixture at one temperature (isothermal nucleic acid
amplification method) and was one of the first RNA
transcription-based amplification methods described. NASBA
normally offers a simple and rapid alternative to PCR for
nucleic acid amplification, and is capable of yielding an
RNA amplification of·a billion fold in 90 minutes. With

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respect to other amplification systems such as the PCR
technique, the ability of NASBA to homogeneously and
isothermally amplify RNA analytes extends its application
range from viral diagnostics to the indication of biological
5   activities such as gene expression and cell viability. NASBA
technology is discussed, for example, in Nature volume 350
pages 91 and 92. Nucleic acid amplification in NASBA is
accomplished by the concerted enzyme activities of AMV
reverse transcriptase, Rnase H, and T7 RNA polymerase,
10 together with a primer pair, resulting in the accumulation
of mainly single-stranded RNA that can readily be used for
detection by hybridization methods. The application of an
internal RNA standard to NASBA results in a quantitative
nucleic acid detection method with a dynamic range of four
15 logs but which needed six amplification reactions per
quantification. This method is improved dramatically by the
application of multiple, distinguishable, internal RNA
standards added in different amounts and by
electrochemiluminesence (ECL) detection technology. This
20 one-tube quantitative (Q) NASBA needs only one step of the
amplification process per quantification and enables the
addition of the internal standards to the clinical sample in
a lysis buffer prior to the actual isolation of the nucleic
acid. This approach has the advantage that the nucleic acid
25 isolation efficiency has no influence on the outcome of the
quantitation, which in contrast to methods in which the
internal standards are mixed with the wild-type nucleic acid
after its isolation from the clinical sample. Quantitative
NASBA is discussed in Nucleic Acid Research (1998) volume
30 26, pages 2150-2155. Post-NASBA product detection, however,
can still be a labour-intensive procedure, normally
involving enzymatic bead-based detection and

electrochemiluminescent (ECL) detection or fluorescent
correlation spectrophotometry. However, as these
methodologies are heterogeneous or they require some
handling of sample or robotic devices that are currently not
5   cost-effective they are relatively little used for high-
throughput applications. A homogeneous procedure in which
product detection is concurrent with target amplification by
the generation of a target-specific signal would facilitate
large-scale screening and full automation. Recently, a
10   novel nucleic acid detection technology, based on probes
(molecular beacons) that fluoresce only upon hybridization
with their target, has been introduced.

     Fluidics is the science of liquid flow in, for example,
15   tubes. For microfabricated devices, flow of a fluid through
the one or more sets of micro or nano sized reaction
chambers is typically achieved using a pump such as a
syringe, rotary pump or precharged vacuum or pressure source
external to the device. Alternatively, a micro pump or
20   vacuum chamber, or lamb wave pumping elements may be
provided as part of the device itself. Other combinations
of flow control elements including pumps, valves and
precharged vacuum and pressure chambers may be used to
control the flow of fluids through the reaction chambers.
25   Other mechanisms for transporting fluids within the system
include electro-osmotic flow. The accurate manipulation of
nanolitre volumes of a fluid sample using such techniques
can be problematic and complicated.

30   The present invention seeks to address at least some of
the problems of the prior art.

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The present invention provides a microfabricated device for fragmenting nucleic acids present in a fluid sample, the device comprising an inlet port, a fragmentation cell, and an outlet port downstream from said inlet port, said cell
5   being in fluid communication with said ports, and wherein said outlet port is dimensioned to impede the flow of a fluid sample out of said cell so as to effect shearing of nucleic acids molecules therein.

10   DNA and/or RNA breaks under mechanical force when pumped through a narrow orifice, due to rapid stretching of the molecule. A pressure-driven flow can lead to a shear force, which leads to fragmentation of the nucleic acids. DNA and/or RNA typically breaks in the middle of the strand.
15   Repetitive shearing results in smaller fragments. The final fragment size will depends on a number of factors including the flow rate, the orifice cross-section (at the inlet and outlet), the number of shearing steps per run, and the initial DNA/RNA length.
20

Advantageously, the width of the fragmentation cell abruptly decreases at the outlet port. This achieves a high velocity gradient in the region adjacent the outlet port.

25   The outlet port typically comprises a constriction, preferably having a width in the range of from 1 to 100 μm, more preferably from 5 to 50 μm.

The fragmentation cell typically comprises a chamber
30   having a bottom wall in which is formed the outlet port, the bottom wall being generally perpendicular to the direction of flow of fluid through the outlet port. The outlet port

is preferably formed in approximately the middle (i.e. the mid-point of the width) of the bottom wall.

In a preferred embodiment, the fragmentation cell has the shape of an irregular polygon (preferably an irregular hexagon) with an essentially straight bottom wall in which the outlet port is formed at approximately the mid point (i.e. the mid-point of the width), and wherein the bottom wall is substantially perpendicular to the longitudinal axis of the outlet port. In this case, the bottom wall is typically adjacent and substantially perpendicular to two lower side wall portions. The upper portions of the side walls preferably taper inwardly to meet the inlet port.

The fragmentation cell will typically have a top wall in which the inlet port is formed, and side walls which extend from the top wall to the bottom wall. The side walls may taper inwardly to meet the inlet port and/or may taper inwardly to meet the outlet port.

The side walls portions next to or adjacent the inlet port advantageously subtend an angle of less than 90 degrees to the longitudinal axis of the inlet port. Such a gradual opening allows for substantially bubble-free filling of the cell.

In a preferred embodiment of the microfabricated device, the fragmentation cell is generally pear shaped with an essentially straight bottom wall in which the outlet port is formed at approximately the mid point (i.e. the mid-point of the width), the bottom wall being substantially perpendicular to the longitudinal axis of the outlet, and

wherein the bottom wall is connected by curved walls to side
walls, which converge or taper inwardly to meet the inlet
port.

5   The device preferably further comprises an obstacle
located in the cell in the direct path between the inlet and
outlet ports. In this case, the space between sides of the
obstacle and sides of the cell preferably defines a
bifurcated path for the fluid sample. Advantageously, the
10   obstacle is shaped so that the flow path of a fluid sample
in a region adjacent the outlet port is substantially
perpendicular to the longitudinal axis of the outlet. In
other words, the direction of fluid flow in the region of
the cell just prior to the outlet is preferably
15   substantially perpendicular to the longitudinal axis of the
outlet (and typically also the longitudinal axis of the
inlet).

        The obstacle may be in the form of a generally
20   triangular obstacle, with its three sides substantially
parallel to the bottom wall and side walls of the cell, the
space between the sides of the obstacle and the sides of the
cell defining a bifurcated path for the fluid sample.

25   The fragmentation cell will typically be asymmetric
about the horizontal axis and substantially symmetric about
the longitudinal axis, the longitudinal axis being
essentially coincident with the direction of flow.

30   The microfabricated device preferably further comprises
an access channel in fluid communication with the inlet
port. The access channel typically connects the inlet port

to the source of the fluid sample and preferably has a
greater flow area than the inlet port. A sample loading
chamber may also be provided to facilitate controlled
loading of a sample into the fragmentation cell.

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The microfabricated device preferably further comprises
collection means in fluid communication with the outlet port
for collecting the fragmented nuclei acids (contained in the
fluid sample).

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The microfabricated device preferably further comprises
mean for effecting flow of a sample into the inlet port,
through the fragmentation cell and out of the outlet port.
Such means may comprise one or more pumps. Alternatively,
15   such means may comprises one or more variable volume
chambers in communication with the inlet port and/or outlet
port, wherein altering the volume of the variable volume
chamber(s) effects and/or restricts flow of a fluid sample
into and/or out of the fragmentation cell. The variable
20   volume chamber typically comprises a flexible membrane
overlying a hollow recess in the substrate.

The microfabricated device will typically comprise a
substrate and an overlying cover, the fragmentation cell
25   being defined by a recess in a surface of the substrate and
the adjacent surface of the cover. The substrate may be
formed from silicon, for example, and the overlying cover
from glass, for example. In this case, the glass cover is
preferably anodically bonded to the silicon substrate,
30   optionally through an intermediate silicon oxide layer
formed on the surface of the substrate.

The microfabricated device preferably comprises at
least first and second fragmentation cells, the outlet port
of the first cell being in fluid communication with the
inlet port of the second cell. Of course, in this manner, a
5   third fragmentation cell may be provided, the outlet port of
the second cell being in fluid communication with the inlet
port of the third cell. Likewise, fourth, fifth, sixth, etc
cells may be provided. Advantageously, the microfabricated
device comprises a plurality (at least two) of serially
10   connected fragmentation cells. Microfluidic implementation
allows linear sequences of multiple fragmentation cells for
repetitive shearing in one run. The size of the outlet port
preferably decreases the further down stream the
fragmentation cell. Thus, the size of the outlet port may
15   progressively decrease from the first fragmentation cell to
the last fragmentation cell (downstream). For example, the
orifice size of the outlet ports can be reduced as degree of
fragmentation increases (eg 10 constrictions from 50 down to
5 µm).
20

A modified design of the fragmentation cell includes an
island or obstacle located in the chamber between the inlet
and outlet ports. In other words, an obstacle is placed in
the path of the jet between the inlet and outlet. Flow is
25   then forced to follow paths around the obstacle, which
prevents jets and circulatory flow from appearing. This
improves the flow pattern for better shearing and bifurcated
flow may be achieved. Furthermore, it has been found that
rounded corners in the design of the cell improve the
30   homogeneity of the flow pattern when compared to structures
containing sharp corners.

The design of the fragmentation cell is preferable such
so as to achieve a substantially perfect laminar, sink-type
flow. It has been found that this type of flow is
advantageous for good nucleic acid fragmentation
5   performance.

The microfabricated device may be used for fragmenting
nucleic acids present in a biological fluid, a dairy
product, an environmental fluid or drinking water.
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The present invention also provides a microfabricated
reaction chamber system for carrying out a nucleic acid
sequence amplification and detection process on a nucleic
acid sample, the system comprising a microfabricated device
15   as herein described. The device and process as herein
described and according to the present invention may also be
used to fragment polymers, such as polysaccharides, and
proteins which may be present in a fluid sample.

20   The present invention also provides an apparatus for
the analysis of biological and/or environmental samples, the
apparatus comprising a device or a system as herein
described. The apparatus may be disposable.

25   The present invention also provides an assay kit for
the analysis of biological and/or environmental samples, the
kit comprising a device or a system as herein described and
means for contacting the sample with the device. The assay
kit may be disposable.
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The present invention also provides a method for DNA
and/or RNA fragmentation based on the application of a

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hydrodynamic shear force to the DNA/RNA molecules, using a device as herein described. The device mimics mesoscopic fragmentation devices (such as described in P. J. Oefner, S. P. Hunicke- Smith, L. Chiang, F. Dietrich, J. Mulligan, and R. W. Davis, Nucleic Acids Research , vol. 24, pp. 3879-3886, 1996), taking advantage of microfluidic assets such as low dead-volume and short processing times. The device enables a 48 kbp lambda DNA sample to be fragmented down to 4 kbp.

Fragmentation may be achieved by passing a sample through alternating, horizontal contractions of the flow path from, say, 100 µm down to 30 µm or smaller. Generally speaking, this layout requires only one lithography and etching step, and is therefore easily fabricated. If larger diameter orifices are incorporated into the channels, in order to prevent clogging, then higher flow rates must be used in order to obtain the same shearing rates. Sequences of progressively shrinking orifices ensure a low risk of clogging at the beginning (approx. 50 µm widths), and higher shear rates towards the end of the channel to achieve smaller fragments (orifices approx. 10 µm wide). Structures having series of orifices of decreasing diameter help alleviate any clogging problems. This is because the larger fragments will have been broken into smaller fragments by the time they arrive at smaller diameter orifices.

The microfabricated device may be used in a system for carrying out any suitable biological or chemical reaction such as, for example, enzyme reactions, immuno reactions, sequencing, hybridisation.

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The nucleic acid sample may be derived from, for example, a biological fluid, a dairy product, an environmental fluids and/or drinking water. Examples include blood, serum, saliva, urine, milk, drinking water, marine water and pond water. For many complicated biological samples such as, for example, blood and milk, it will be appreciated that before one can isolate and purify DNA and/or RNA from bacterial cells and virus particles in a sample, it is first necessary to separate the virus particles and bacterial cells from the other particles in sample. It will also be appreciated that it may be necessary to perform additional sample preparation steps in order to concentrate the bacterial cells and virus particles, i.e. to reduce the volume of starting material, before proceeding to break down the bacterial cell wall or virus protein coating and isolate nucleic acids. This is important when the starting material consists of a large volume, for example an aqueous solution containing relatively few bacterial cells or virus particles. This type of starting material is commonly encountered in environmental testing applications such as the routine monitoring of bacterial contamination in drinking water.

As indicate earlier, the device and process as herein described may be used to fragment polymers, such as polysaccharides, and proteins which may be present in a fluid sample such as a biological fluid, a dairy product, an environmental fluids and/or drinking water.

The system is preferably designed to cater for a sample volume of =< 50 nl, preferably =< 20 nl, more preferably =< 10 nl. Thus the volume of each of the reaction chambers

will typically be =< 50 nl, preferably =< 20 nl, more
preferably <= 10 nl. However, and as will be appreciated,
larger sized chambers may be used, for example chambers
having a volume of 100 to 500 nl.

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An integrated microfabricated reaction chamber system
may be provided with a plurality of devices as described
above, each of which may have a separate outlet port. In
this way a range of different analysis processes may be
10   carried out simultaneously within a single micromachined
device.

The present invention also provides a method for the
manufacture of a microfabricated device as herein described,
15   which method comprises:
(i) providing a substrate having at least one recess in a
surface thereof;
(ii) providing a cover; and
(iii) bonding the cover to the substrate to create at least
20   one fragmentation cell defined by said at least one recess
in said surface of the substrate and the adjacent surface of
the cover. The substrate may be formed from silicon, for
example, and the overlying cover from glass, for example.
In this case, the glass cover is preferably anodically
25   bonded to the silicon substrate, optionally through an
intermediate silicon oxide layer formed on the surface of
the substrate. The recess in the silicon may be formed
using reactive-ion etching. Other materials such as
polymeric materials may also be used for the substrate
30   and/or cover.

Preferably, and in particular if optical observations
of the contents of the cell are required, the overlying
cover is made of an optically transparent substance or
material, such as glass or Pyrex.

The term recess as used herein is also intended to
cover a variety of features including, for example, grooves,
slots, holes, trenches and channels, including portions
thereof.

The system or at least a master version thereof will
typically be formed from or comprise a semiconductor
material, although dielectric (eg glass, fused silica,
quartz, polymeric materials and ceramic materials) and/or
metallic materials may also be used. Examples of
semiconductor materials include one or more of: Group IV
elements (i.e. silicon and germanium); Group III-V
compounds (eg gallium arsenide, gallium phosphide, gallium
antimonide, indium phosphide, indium arsenide, aluminium
arsenide and aluminium antimonide); Group II-VI compounds
(eg cadmium sulphide, cadmium selenide, zinc sulphide, zinc
selenide); and Group IV-VI compounds (eg lead sulphide,
lead selenide, lead telluride, tin telluride). Silicon and
gallium arsenide are preferred semiconductor materials. The
system may be fabricated using conventional processes
associated traditionally with batch production of
semiconductor microelectronic devices, and in recent years,
the production of semiconductor micromechanical devices.
Such microfabrication technologies include, for example,
epitaxial growth (eg vapour phase, liquid phase, molecular
beam, metal organic chemical vapour deposition), lithography
(eg photo-, electron beam-, x-ray, ion beam-), etching (eg

chemical, gas phase, plasma), electrodeposition, sputtering, diffusion doping, ion implantation and micromachining. Non-crystalline materials such as glass and polymeric materials may also be used.

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Examples of polymeric materials include PMMA (Polymethyl methylacrylate), COC (Cyclo olefin copolymer), polyethylene, polypropylene, PL (Polylactide), PBT (Polybutylene terephthalate) and PSU (Polysulfone),
10 including blends of two or more thereof. Hot embossing of such polymeric materials may be used to form the device.

Combinations of a microfabricated component with one or more other elements such as a glass plate or a complementary
15 microfabricated element are frequently used and intended to fall within the scope of the term microfabricated used herein.

The substrate base may be provided with a coating of
20 thickness typically up to 1 µm, preferably less than 0.5 µm. The coating is preferably formed from one or more of the group comprising polyethylene glycol (PEG), Bovine Serum Albumin (BSA), tweens and dextrans. Preferred dextrans are those having a molecular weight of 9,000 to 200,000,
25 especially preferably having a molecular weight of 20,000 to 100,000, particularly 25,000 to 75,000, for example 35,000 to 65,000). Tweens (or polyoxyethylene sorbitans) may be any available from the Sigma Aldrich Company. PEGs are preferred as the coating means, either singly or in
30 combination. By PEG is embraced pure polyethylene glycol, i.e. a formula $HO-(CH_2CH_2O)_n-H$ wherein n is an integer, whereby to afford a PEG having molecular weight of from

typically 200 - 10,000, especially PEG 1,000 to 5,000; or chemically modified PEG wherein one or more ethylene glycol oligomers are connected by way of homobifunctional groups such as, for example, phosphate moieties or aromatic

5   spacers. Particularly preferred are polyethylene glycols known as FK108 (a polyethylene glycol chain connected to another through a phosphate); and the PEG sold by the Sigma Aldrich Company as product P2263. The above coatings applied to the surfaces of the cell/chamber, inlets,

10   outlets, and/or channels can improve fluid flow through the system. In particular, it has been found that the sample is less likely to adhere or stick to such surfaces. PEG coatings are preferred.

15   The device/system will typically be integrally formed. The device/system may be microfabricated on a common substrate material, for example a semiconductor material as herein described, although a dielectric substrate material such as, for example, glass or a ceramic material could be

20   used. The common substrate material is, however, preferably a plastic or polymeric material and suitable examples are given above. The system may preferably be formed by replication of, for example, a silicon master. This may be achieved, for example, by hot embossing of a polymeric

25   material.

        The microfabricated device/system may be designed to be disposable after it has been used once or for a limited number of times. This is an important feature because it

30   reduces the risk of contamination.

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The microfabricated device/system may be incorporated into an apparatus for the analysis of, for example, biological fluids, dairy products, environmental fluids and/or drinking water. Again, the apparatus may be designed
5 to be disposable after it has been used once or for a limited number of times.

The microfabricated system/apparatus may be included in an assay kit for the analysis of, for example, biological
10 fluids, dairy products, environmental fluids and/or drinking water, the kit further comprising means for contacting the sample with the device. Again, the assay kit may be designed to be disposable after it has been used once or for a limited number of times.
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The microfabricated system as herein described is also intended to encompass nanofabricated devices.

For a silicon or semiconductor master, it is possible
20 to define by, for example, etching or micromachining, one or more of variable volume chambers, microfluidic channels, reaction chambers and fluid interconnects in the silicon substrate with accurate microscale dimensions (deep reactive-ion etching (DRIE) is a preferred technique). A
25 plastic replica may then be made of the silicon master. In this manner, a plastic substrate with an etched or machined microstructure may be bonded by any suitable means (for example using an adhesive or by heating) to a cover thereby forming the enclosed fragmentation cell(s), inlet(s),
30 outlet(s) and connecting channel(s).

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The present invention may also be used in conjunction
with a microfabricated device for nucleic acid extraction,
which device preferably comprises an extraction cell having
an inlet port for introducing a sample (eg from the
5   fragmentation cell) and an outlet port downstream from said
inlet port for withdrawing extracted nuclei acids, wherein
said extraction cell is at least partially filled with
silica beads or particles. Such a device may be used for
nucleic acid extraction. This aspect of the present
10   invention is based to an extent on the finding that NA binds
to silica surfaces in the presence of chaotropic agents
The extraction cell may have any suitable shape and
configuration but will typically be in the form of a channel
or a chamber. The extraction cell preferably further
15   comprises one or more sets of electrodes adjacent the silica
beads or particles for collecting and/or preconcentrating
the eluted nucleic acids. Said one or more sets of
electrodes preferably comprise platinum electrodes. Means
may therefore be provided for applying a potential
20   difference accross the electrodes. The integration of
electrodes may be used to reversibly collect and
preconcentrate the eluted NA on-chip. Thus, this aspect of
the present invention enables combined nucleic acid
extraction and enrichment to be achieved.
25

The optional microfabricated device for nucleic acid
extraction will typically comprise a substrate and an
overlying cover, the extraction cell being defined by a
recess in a surface of the substrate and the adjacent
30   surface of the cover. The substrate is preferably formed
from silicon or poly(dimethylsiloxane) (PDMS). PDMS
channels may be fabricated by replica molding using a 2-

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layer SU-8 master. Dry, 15-35 μm silica particles can be
packed into the channels using a vacuum. Platinum
electrodes can be patterned on a Pyrex wafer by a lift-off
process.

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The present invention also provides a process for
fragmenting nucleic acids present in a fluid sample, the
process comprising:
(a)   providing a microfabricated device as herein described;
10   (b)   providing a fluid sample comprising nucleic acids;
(c)   injecting the fluid sample into the inlet port of said
device, through the fragmentation cell and out of the outlet
port; and
(d)   collecting the thus fragmented sample at the outlet
15   port.

The process preferably further involves a nucleic acid
sequence amplification and detection process on the
fragmented nucleic acid sample.

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The present invention will now be described, by way of
example, with reference to the accompanying drawings, of
which:-

25   Figures 1(a) and (b) are close-up views of a
microfabricated device according to the present invention
formed from silicon.

Figures 2(a) and (b) shows the dependence of fragment
30   size on shear rate for a chip with 10 constrictions of
approx. 15 μm width.

Figures 3 (a), (b) and (c) shows an alternative
structure for the fragmentation chamber in which an island
or obstacle is inserted in the chamber to effect bifurcated
flow. (a) Close-up of cell with approx. 10 µm wide orifices,
5 the wide of the channels are approx. 100 µm, and the flow
direction is from left to right. (b) View of a series of
device with five orifices, each approx. 25 µm wide. To the
left and right, approx. 400 µm wide access channels are
visible. (c) Close-up of a approx. 10 µm wide orifice.
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Figures 4 (a), (b) and (c) provide a comparison between
the fragmentation chambers according to Figure 1 (a) and
Figure 3 (a). Figure 4(a) corresponds to a cell similar to
the one shown in Figure 1(a). Figure 4(b) corresponds to a
15 cell similar to the one shown in Figure 3(a). Figure 4(c)
is a plot of fragment size against shear rate for the two
structures.

Figures 1(a) and (b) show a close-up view of one
20 fragmentation cell (or shearing unit) in a microfabricated
silicon device, and a series of consecutive cells with
monotonically decreasing orifices, respectively.

Figure 1 (a) shows a scanning electron micrograph (SEM)
25 of a fragmentation cell 1 (or shearing unit), one in a
connected series made by deep reactive-ion etching (DRIE) in
silicon. The constriction outlet 10 (approx. 75 µm long) is
designed to have an abrupt change in cross-section from
large to small in the flow direction. At the chamber inlet
30 20, a gradual opening has been found to help avoid air
bubbles being trapped in the structure. The constriction

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width (i.e. the width of the outlet and inlet) is approx. 25 µm and the feature depths approx. 50 µm.

5 The shape of fragmentation cell 1 may be described as an irregular hexagon with an essentially straight bottom wall 5 in which the outlet 10 is formed at approximately the mid point. It can be seen that the bottom wall 5 is substantially perpendicular to the longitudinal axis of the outlet 10 (and the direction of flow). Thus, the bottom

10 wall 5 subtends an angle of approximately 90 degrees to the longitudinal axis of the outlet 10 (and the direction of flow). The bottom wall 5 is adjacent and substantially perpendicular to two lower side wall portions 15a and 15b. The upper portions 15c and 15d of the side walls taper

15 inwardly to meet the inlet 20 at the top of the cell 1. Thus, upper side walls portions 15c and 15d each subtend an angle of less than 90 degrees to the longitudinal axis of the inlet (and the direction of flow). It can be seen, however, that the uppermost side wall portions 15e and 15f

20 immediately adjacent the inlet 20 subtend an angle of approximately 90 degrees to the longitudinal axis of the inlet 10 (and the direction of flow). It can also be seen that the cell 1 is asymmetric about the horizontal axis and substantially symmetric about the longitudinal axis (the

25 longitudinal axis is essentially coincident with the direction of flow).

Figure 1 (b) shows a SEM of the device with 10 constrictions with decreasing widths from approximately 50

30 to 5 µm. Feature depths were either approximately 50 or 75 µm. This is an example of a device with a series of constrictions of progressively decreasing width. Device

clogging is reduced, since the larger DNA fragments passing
through the wider constrictions at the beginning are reduced
in size by the time they reach the smaller constrictions.
At these later, smaller channels, the higher shear rates
required for breaking the smaller fragments can be
generated.

The microfabricated device will generally be part of a
chip. The device comprises a substrate with the desired
microstructure formed in its upper surface. The substrate
may be silicon, for example, or a plastic substrate formed
by replication of a silicon master. The substrate is bonded
at its upper surface to a cover, thereby defining a series
of fragmentation cells, inlets, outlets, and channels. The
cover may be formed from plastic or glass, for example. The
cover is preferably transparent and this allows observation
of the fluid. In general, the device is preferably
fabricated by deep reactive-ion etching (DRIE) of silicon
for high aspect ratio constrictions, followed by anodic
bonding of a glass cover. For example, fabrication of the
devices shown in the Figures involved the following steps:
(1) silicon substrate; (2) negative photoresist (eg MAN 420,
approx. 2.7 µm thick) defining desired microstructure; (3)
deep reactive-ion etching (approx. 50-75 µm deep);(4)
photoresist removal; (5) thermal oxidation (200 nm); and (6)
anodic bonding to a drilled Pyrex 7740 glass cover plate
(approx. 800 V, 360°C).

With reference to Figures 2(a) and (b), a syringe pump
was used to pump sample (48 kbp lambda DNA (67 mg/mL in 10
mM Tris-HCl/5 mM NaCl/0.1 mM EDTA, pH 7.0)) through the
chips. The electropherograms in Figure 2(a) show that there

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is a shift towards smaller fragment sizes as flow rate is
increased, as expected. In fact, the sharp peaks at about
35 and 90 seconds are due to size marker DNA molecules
corresponding to 50 and 17000 bp, respectively. For a shear
5   rate of $3 \times 10^6 s^{-1}$ (1000 mL/min), fragment size could be
reduced to about 4000 bp (value at the electropherogram peak
maximum). A size distribution of 1370 - 9780 bp was
obtained at this flow rate, which is somewhat larger than
the 2-fold size distribution expected if fragments had been
10   broken ideally at their midpoints each time. Figure 2(b)
gives fragment size at peak maximum as a function of shear
rate, and clearly shows the decrease in DNA fragment size as
shear rate increases.

15   Figure 3 shows an alternative structure for the
shearing chamber which sees the insertion of an island or
obstacle in the chamber (approximately in the centre) to
effect bifurcated flow. In other words, an obstacle is
placed in the path of the jet between the inlet and outlet.
20   Flow is then forced to follow paths around the obstacle,
which prevents jets and circulatory flow from appearing.
Furthermore, the rounded corners of the design have been
found to improve the homogeneity of the flow pattern when
compared to structures containing sharp corners. The etch
25   depth is approximately 50 μm. The design achieves a
substantially perfect laminar, sink-type flow. It has been
found that this type of flow is advantageous for good
nucleic acid fragmentation performance.

30   Comparing Figure 3 to Figure 1, the shape of the
fragmentation cell 30 may be described as pear shaped with
an essentially straight bottom wall 35 in which the outlet

40 is formed at approximately the mid point. It can be seen
that the bottom wall 35 is substantially perpendicular to
the longitudinal axis of the outlet 40 (and the direction of
flow). Thus, the bottom wall 35 subtends an angle of

5   approximately 90 degrees to the longitudinal axis of the
outlet 40 (and the direction of flow). The bottom wall 35
is connected by curved walls 45a and 45b to side walls 50a
and 50b, which taper inwardly to meet the inlet 60 at the
top of the cell. Thus, the side walls 50a and 50b each

10   subtend an angle of less than 90 degrees to the longitudinal
axis of the inlet (and the direction of flow). A generally
triangular obstacle 70 is located in the cell, with its
three sides substantially parallel to the bottom wall 35 and
side walls 50a and 50b. The space between the sides of the

15   obstacle and the sides of the cell defines a bifurcated path
for the fluid sample.

Figures 4 (a), (b) and (c) provide a comparison between
a fragmentation chamber design based on Figure 1 (a) and one

20   based on Figure 3 (a) (Figure 4(a) corresponds to a cell
similar to the one shown in Figure 1(a), while Figure 4(b)
corresponds to a cell similar to the one shown in Figure
3(a)). Figure 4(c) gives fragment size at peak maximum as a
function of shear rate, and clearly shows the decrease in

25   DNA fragment size as shear rate increases, and also shows
the difference in flow dynamics between the two structures
results in different fragmentation performance. In
particular, the design shown in Figure 4(b) results in
smaller fragments compared with the design shown in Figure

30   4(a).

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The present invention also provides a device which includes a microfabricated device/system as herein described, together and preferably in fluid communication with one or more of:

5 (A) means for filtering a sample prior to carrying out the method according to the present invention, for example to substantially remove particles contained in the sample which are larger in size that bacteria particles; and/or

(B) means for separating virus particles and/or bacterial

10 cells from the other particles in a sample prior to carrying out the method according to the present invention; and/or

(C) means for concentrating bacterial cells and/or virus particles, i.e. to reduce the volume of starting material, prior to carrying out the method according to the present

15 invention; and/or

(D) means for breaking down the bacterial cell wall or virus protein coating and isolate nucleic acids prior to carrying out the method according to the present invention.

20   A microfabricated device embodying the invention may form an integral part of a larger microfabricated analysis device constructed as a single unit and containing, for example, apparatus for carrying out various sample preparation steps, and containing the various reagents

25 required to carry out the sample preparation steps. Such a microfabricated analysis device could contain some or all of the control and data analysis circuitry required for its operation.

30 **Example**

General

Chips were fitted into chip holders where polyetherketone (PEEK) tubing could be connected to the microchannels in the Si-device. The fragmentation of the DNA/RNA was done manually using a Hamilton-syringe. Before fragmentation the device was flushed with 100 µl pure water (Sigma W4502) to remove any air in the system. The fragmentation was done by pumping 100 µl of a HPV 45 positive sample. The sample was pretreated as follows: 100 µl HPV 45 positive sample in methanol-buffer (PreservCyt, CYTYC) was sentrifugated for 5 min at 800 rpm. The pellet was then resuspended in 900 µl water (Sigma). 100 µl of this solution was then used in the fragmentation device.

Specification of the fragmentation device

A device as herein described and having a fragmentation cell of the type shown in Figure 4(a) was used. The orifices width (or constriction) is approximately 20 µm, while the orifice length is approximately 50 µm.

Experimental procedure:

1. Pump 100 µl pure water through the device to remove any air (Sigma W4502, RNase- and DNase free).

2. Pump 100 µl of the sample.

3. Pump 100 µl of RNa- later, non-diluted (Ambion 7022).

4. Collect sample (extract) and the RNa-later and make analysis (NASBA).

Results: NASBA (Nucleic Acid Sequence Based Amplification)

A positive centrifugated HPV 45 sample was pumped through the fragmentation device and the outcome was analysed in NASBA the same day. The sample-extract was tested non-diluted with PreTect HPV Proofer kit (NorChip).

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The NASBA-result showed a 6.14 times increase in the signal for HPV 45 from the startlevel. Negative control (pure Sigma-water) showed no increase in signal for HPV 45. Positive control for HPV 45 (artificial designed HPV 45 DNA-oligo, diluted 1 to a million) showed 6.99 times increase in signal.

Results: RNase-activity

The sample extract was also tested for RNase-activity with the RNase Alert Lab Test Kit (Ambion 1964). The sample extract showed no Rnase activity compared to a negative and positive RNase control.

Implementation of DNA shearing in microfluidic devices is advantageous for a number of reasons. Microchannel geometries incorporating small (=< 50 µm), highly reproducible constrictions are possible, allowing generation of high shear rates at comparatively low flow rates (in the order of µL/min rather than mL/min). This, combined with smaller device volumes, facilitates processing of small samples. Multiple shearing steps can be accomplished by inserting a number of constrictions into a microchannel. In this way, sample does not need to be continually recirculated through the same constriction, and flow system dead volume can be substantially reduced.